abcam

Product datasheet

Anti-BubR1 antibody [EPR20652] - BSA and Azide free ab251514

Recombinant RabMAb

8 Images

Overview

Product name	Anti-BubR1 antibody [EPR20652] - BSA and Azide free
Description	Rabbit monoclonal [EPR20652] to BubR1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
General notes	ab251514 is the carrier-free version of **ab209998**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20652
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab251514 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 120 kDa (predicted molecular weight: 119 kDa).
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

Target

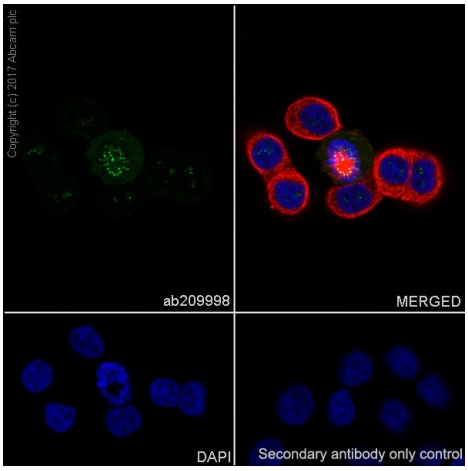
Function Essential component of the mitotic checkpoint. Required for normal mitosis progression. The mitotic checkpoint delays anaphase until all chromosomes are properly attached to the mitotic spindle. One of its checkpoint functions is to inhibit the activity of the anaphase-promoting complex/cyclosome (APC/C) by blocking the binding of CDC20 to APC/C, independently of its kinase activity. The other is to monitor kinetochore activities that depend on the kinetochore motor CENPE. Required for kinetochore localization of CENPE. Negatively regulates PLK1 activity in interphase cells and suppresses centrosome amplification. Also implicated in triggering apoptosis in polyploid cells that exit aberrantly from mitotic arrest. May play a role for tumor suppression.

Tissue specificity Highly expressed in thymus followed by spleen. Preferentially expressed in tissues with a high mitotic index.

Involvement in disease Note=Defects in BUB1B are associated with tumor formation.
Defects in BUB1B are the cause of premature chromatid separation trait (PCS) [MIM:176430]. PCS consists of separate and splayed chromatids with discernible centromeres and involves all or most chromosomes of a metaphase. It is found in up to 2% of metaphases in cultured lymphocytes from approximately 40% of normal individuals. When PCS is present in 5% or more of cells, it is known as the heterozygous PCS trait and has no obvious phenotypic effect, although some have reported decreased fertility. Inheritance is autosomal dominant.
Defects in BUB1B are the cause of mosaic variegated aneuploidy syndrome (MVA)

	[MIM:257300]. MVA is a severe autosomal recessive developmental disorder characterized by mosaic aneuploidies, predominantly trisomies and monosomies, involving multiple different chromosomes and tissues. The proportion of aneuploid cells varies but is usually more than 25% and is substantially greater than in normal individuals. Affected individuals typically present with severe intrauterine growth retardation and microcephaly. Eye anomalies, mild dysmorphism, variable developmental delay, and a broad spectrum of additional congenital abnormalities and medical conditions may also occur. The risk of malignancy is high, with rhabdomyosarcoma, Wilms tumor and leukemia reported in several cases. MVA is caused by biallelic mutations in the BUB1B gene.
Sequence similarities	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. BUB1 subfamily. Contains 1 BUB1 N-terminal domain. Contains 1 protein kinase domain.
Domain	The D-box targets the protein for rapid degradation by ubiquitin-dependent proteolysis during the transition from mitosis to interphase. The BUB1 N-terminal domain directs kinetochore localization and binding to BUB3.
Post-translational modifications	Proteolytically cleaved by caspase-3 in a cell cycle specific manner. The cleavage might be involved in the durability of the cell cycle delay. Caspase-3 cleavage is associated with abrogation of the mitotic checkpoint. The major site of cleavage is at Asp-610. Acetylation at Lys-250 regulates its degradation and timing in anaphase entry. Ubiquitinated. Degradated by the proteasome. Sumoylated by SUMO2 and SUMO3. The sumoylation mediates the association with CENPE at the kinetochore. Autophosphorylated in vitro. Intramolecular autophosphorylation is stimulated by CENPE. Phosphorylated during mitosis and hyperphosphorylated in mitotically arrested cells. Phosphorylation at Ser-670 and Ser-1043 occurs at kinetochores upon mitotic entry with dephosphorylation at the onset of anaphase.
Cellular localization	Cytoplasm. Nucleus. Chromosome > centromere > kinetochore. Cytoplasm > cytoskeleton > centrosome. Cytoplasmic in interphase cells. Associates with the kinetochores in early prophase. Kinetochore localization requires BUB1, PLK1 and CASC5.

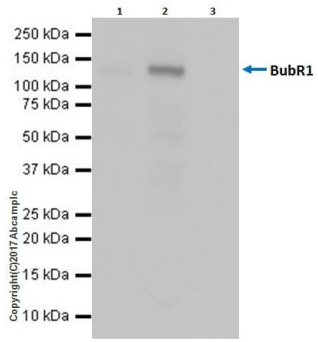
Images



Immunocytochemistry/ Immunofluorescence - Anti-BubR1 antibody [EPR20652] - BSA and Azide free (ab251514)

This data was developed using **ab209998**, the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HT-29 (human colorectal adenocarcinoma cell line) cells labeling BubR1 with **ab209998** at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing specific staining on centromeres of HT-29 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Immunoprecipitation - Anti-BubR1 antibody [EPR20652] - BSA and Azide free (ab251514)

This data was developed using **ab209998**, the same antibody clone in a different buffer formulation.

BubR1 was immunoprecipitated from 0.35 mg of HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with **ab209998** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab209998** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1/1000 dilution.

Lane 1: HeLa whole cell lysate 10 Âµg (Input).

Lane 2: **ab209998** IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab209998** in HeLa whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 1 second.

All lanes : Anti-BubR1 antibody [EPR20652] (**ab209998**) at 1/1000 dilution

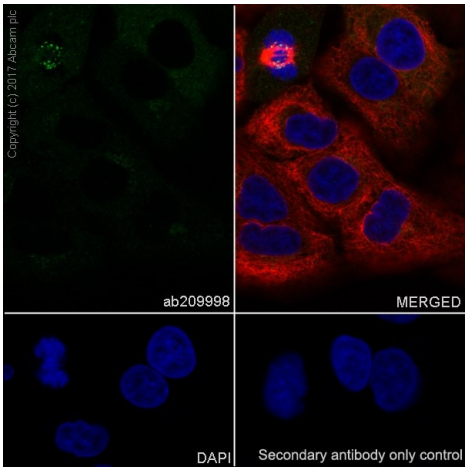
All lanes :

Secondary

All lanes : VeriBlot for IP Detection Reagent (HRP) (**ab131366**)

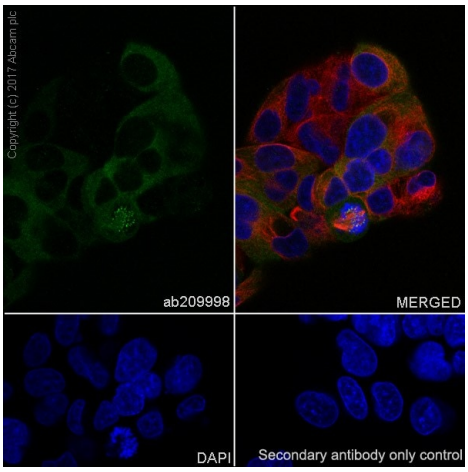
Observed band size: 120 kDa

Exposure time: 1 second



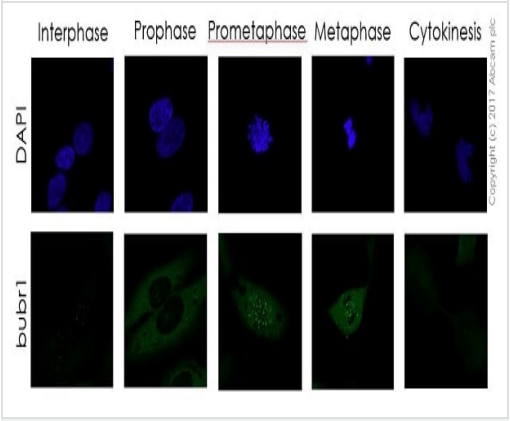
Immunocytochemistry/ Immunofluorescence - Anti-BubR1 antibody [EPR20652] - BSA and Azide free (ab251514)

This data was developed using **ab209998**, the same antibody clone in a different buffer formulation.Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A549 (human lung carcinoma cell line) cells labeling BubR1 with **ab209998** at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing specific staining on centromeres of A549 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



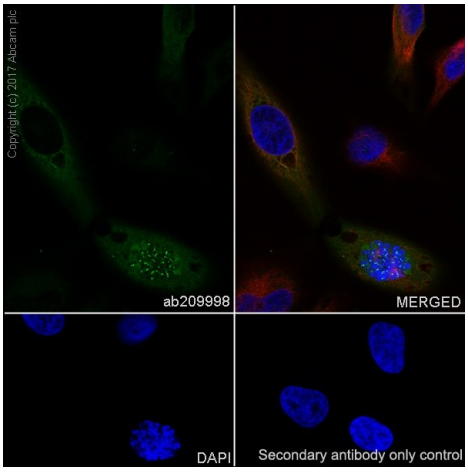
Immunocytochemistry/ Immunofluorescence - Anti-BubR1 antibody [EPR20652] - BSA and Azide free (ab251514)

This data was developed using **ab209998**, the same antibody clone in a different buffer formulation.Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HepG2 (human liver hepatocellular carcinoma cell line) cells labeling BubR1 with **ab209998** at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing specific staining on centromeres of HepG2 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-BubR1 antibody [EPR20652] - BSA and Azide free (ab251514)

This data was developed using **ab209998**, the same antibody clone in a different buffer formulation.Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling BubR1 with **ab209998** at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing subcellular localization of BubR1 changes through mitosis of HeLa cell line (PMID: 25833949). The nuclear counter stain is DAPI (blue).



Immunocytochemistry/ Immunofluorescence - Anti-BubR1 antibody [EPR20652] - BSA and Azide free (ab251514)

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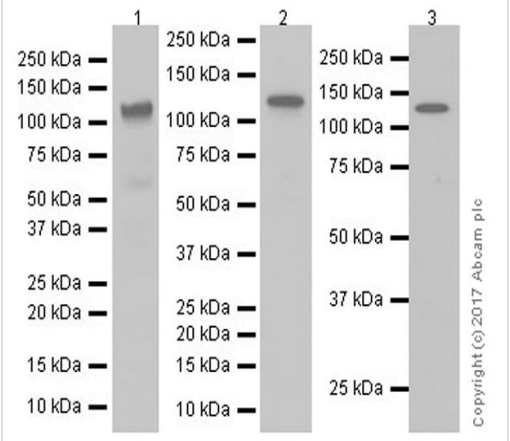
-ve control: Secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary at 1/1000 dilution.



Western blot - Anti-BubR1 antibody [EPR20652] - BSA and Azide free (ab251514)

All lanes : Anti-BubR1 antibody [EPR20652] (**ab209998**) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : Raji (human Burkitt's lymphoma cell line) whole cell lysate
Lane 3 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Performed under reducing conditions.

Predicted band size: 119 kDa
Observed band size: 120 kDa

This data was developed using **ab209998**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1: 10 seconds; Lane 2: 3 seconds; Lane 3: 3 minutes.

Positive BubR1 detection by western blot is dependent on the number of mitotic cells in the sample and may need optimization for some cell and tissue lysates.

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